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ANNEX

CLAIMS

1. Polypeptides having antifreeze activity which can be obtained from carrots and which have an apparent molecular weight on SDS-PAGE of 36 kDa and isoforms or derivatives thereof which still possess antifreeze activity.

2. Polypeptides having antifreeze activity comprising one or more fragments (A-E) of the amino acid sequence as follows:

(A) LEU-PRO-ASN-LEU-PHE-GLY-LYS

(B) ILE-PRO-GLU-GLU-ILE-SER-ALA-LEU-LYS

(C) LEU-THR-X-LEU-ASP-LEU-SER-PHE-ASN-LYS

(D) SER-LEU-ARG-LEU-SER-SER-THR-SER-LEU-SER-GLY-PRO-VAL-PRO-LEU-PHE-PHE-PRO-GLN-LEU-X-LYS

(E) X-X-GLY-VAL-ILE-PRO-X-GLN-LEU-SER-THR-LEU-PRO-ASN-LEU-LYS

and isoforms or derivatives thereof which still possess antifreeze activity.

3. Polypeptides having antifreeze activity comprising the fragments (A-E) of claim 2.

4. Polypeptides having antifreeze activity having an amino acid sequence as represented in Listing 1 and isoforms and derivatives thereof which still possess antifreeze activity.

5. An isolated nucleic acid sequence encoding the antifreeze polypeptide of one or more of ~~claims 1-4~~ Claim 1 and alleles thereof encoding polypeptides which still possess antifreeze activity.

6. An isolated nucleic acid sequence corresponding to the gene sequence of Listing I and alleles thereof encoding polypeptides which still possess antifreeze activity .

7. Method of obtaining polypeptides according to one or more of ~~claims 1-4~~ Claim 1 whereby the polypeptide is isolated from cold-acclimatised carrots.

8. Method of obtaining polypeptides according to one or more of ~~claims 1-4~~ Claim 1, whereby the polypeptide is expressed by a genetically modified organism.

9. Method according to claim 8, whereby the organism is a micro-organism, a plant or a cell culture.

10 An antibody capable of specifically binding the polypeptide of ~~claim~~ 1, ~~2, 3 or 4~~.

11. A polypeptide which has antifreeze activity that is immunologically related to the polypeptide of claim 1, 2, 3 or 4 as determined by its cross reactivity with an antibody of claim 10.

12. Food product comprising a polypeptide of ~~claim~~ 1, ~~2, 3, 4 or 11~~ with the proviso that the food product is not a carrot.

13. Food product of claim 12 being a frozen confectionery product or a frozen vegetable.

14. Method of producing a food product comprising an antifreeze polypeptide according to ~~one or more~~ ~~claims~~ 1, ~~2, 3 or 4~~, comprising the steps of

(a) adding to the food product a composition comprising said antifreeze polypeptide; or

(b) in situ production of said antifreeze polypeptide.

15. Use of the polypeptide of ~~claims~~ 1, ~~2, 3 or 4~~ for increasing the frost tolerance of plants.

16. Micro-organisms, cell line or plant capable of expressing the polypeptide of claims 1, 2, 3 or 4, with the proviso that the plant is not an unmodified carrot plant.

The above data provides the proof that the protein purified from carrot and the corresponding cDNA represent an active AFP.